PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 4402PTWO/er	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/EP2004/051758	International filing date *(day/month/year)* 10.08.2004	Priority date *(day/month/year)* 12.08.2003
International Patent Classification (IPC) or national classification and IPC C12N5/00, C12N5/06, A61K35/12		
Applicant ISTITUTO NAZIONALE PER LE MALATTIE INFET... et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 7 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 3 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☒	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
10.06.2005	09.08.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer van Heusden, M Telephone No. +49 89 2399-8145

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No. PCT/EP2004/051758

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages	
1-13	as originally filed
Claims, Numbers	
1-30	received on 12.07.2005 with letter of 12.07.2005
Drawings, Sheets	
1/1	as originally filed

   ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/EP2004/051758

Box No. II Priority

1. ☒ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:
   ☒ copy of the earlier application whose priority has been claimed (Rule 66.7(a)).
   ☐ translation of the earlier application whose priority has been claimed (Rule 66.7(b)).

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rule 64.1). Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

   see separate sheet

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	10,13,23-30
	No:	Claims	1-9,11,12,14-22
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-30
Industrial applicability (IA)	Yes:	Claims	1-30
	No:	Claims	

2. Citations and explanations (Rule 70.7):

   see separate sheet

Additional remarks to section II:

1. The documents mentioned in this report are numbered as in the International Search Report (ISR), i.e. D1 corresponds to the first document of the ISR etc.

2. The priority document pertaining to the present application was not available at the time of establishing this report. Hence, the current assessment is based on the assumption that all claims enjoy priority rights from the filing date of the priority document. If it later turns out that this is not correct, the document by Bordoni (cited as D1 in the International Search Report) could become relevant to assess whether the subject matter of claims 1-29 of the present application satisfies the criteria set forth in Article 33(1) PCT.

Additional remarks to section V:

1. **Novelty and inventive step** (Article 33(2)(3) PCT)

1.1 The present application relates to a culture medium conditioned by immortalized, non-transformed and differentiated hepatocytes, e.g. MMH cells (met murine hepatocytes).

1.2 Interpretation of the claims:
Claims 1-11 relate to the culture medium itself (not to its use). Claim 1 states that the medium is free from conditioning cells **when** used for maintenance, proliferation and differentiation of adult mammalian cells. Thus claim relates to a medium that is free from conditioning cells, claim 1 does not relate to the use of said medium.
The subject matter of claims 4-6 refers to the intended use of the culture medium and therefore does not add any technical feature characterizing the culture medium itself.
In this context, it is noted that claim 5 refers to non human embryonic stem cells. Embryonic stem cells cannot be adult mammalian cells. Therefore the subject matter of claim 5 contradicts with new claim 1 (adult mammalian cells). The same objection applies to claim 25.

The subject matter of claim 11 relates to a culture medium according to claims 4-6,

which culture medium is the same as that according to claims 1-3. This is because claims 4-6 only relate to an intended use, not to the use itself (claims 4-6 are product claims). Claim 11 refers to the culture with mammalian cells in order to further condition the MMH-conditioned medium. This is also considered as an intended use and therefore does not constitute a technical feature characterizing the culture medium. Thus it follows that the culture medium according to claim 11 still has the same technical features as that of claims 1-3.

Claim 12 relates to a process for the production of a culture medium. This process comprises two steps: (1) incubating immortalized untransformed hepatocytes ... in a culture medium and (2) separating said hepatocytes from the medium (at the indicated time point: before using the medium for culturing other cells). Claim 12 does not include the step of using the medium for culturing cells.

Claim 30 relates to a kit comprising the culture medium according to claims 1-11 together with instructions for use. This claim is a product claim. The fact that the kit (the culture medium) is suitable for a certain application (maintenance of adult mammalian cells) is not considered a technical feature characterizing the kit (=product) itself.

1.3 Document D2 discloses the use of murine MMH cells as feeder cells and the co-culture of these MMH cells with fetal liver hematopoietic cells (progenitors). D2 also mentions on p.1653 (column 1, paragraph 2) that fetal hematopoietic progenitor cells were cultured in MMH-conditioned medium (based on Iscove's medium , see p. 1646, column 1, §3). The MMH cells grow in monolayers and require collagen I for attachment (p. 1647, column 2., §2). Said culture results in differentiation of progenitor cells but not in the maintenance of progenitor cells. Thus by disclosing culture in MMH-conditioned medium, D2 inherently discloses culture medium conditioned by MMH cells. MMH cells are inherently genetically modified due to the method by which they have been provided (see document D3 and D4). Therefore D2 anticipates the subject matter of claims 1-9 and 11-12, 14-16.

1.4 The subject matter of claims 17-22 relates to mammalian cells treated with the conditioned culture medium, or pharmaceutical compositions comprising said

mammalian cells. Said mammalian cells, defined as a product by process, do not appear to differ from any known mammalian cells cultured in any other media, or from mammalian cells co-cultured with MMH cells as disclosed in D2 and subsequently purified. Therefore document D2, as well as any adult mammalian cell known in the art, anticipates the subject matter of claims 17-22.

1.5 Claims 10 and 13 do not appear to include any additional matter that could render them inventive as such. Claim 30 also does not involve an inventive step: no inventive activity would be required to provide a kit containing the culture medium as disclosed in D2.

1.6 The subject matter of claims 23-29 relates to the **use** of the conditioned medium according to claims 1-11 for growing, expanding, maintaining and differentiating isolated adult mammalian cells in vitro. The applicant has argued in the letter dated 12.07.05 that document D2 only discloses the culturing of fetal liver hematopoietic cells. To render the claimed subject matter novel over D2 the applicant has restricted the claims to the culturing of adult mammalian cells. The ED, however, considers that D2 also refers to adult cells: on p. 1647 (column 2, § 3) D2 discloses that similar results were obtained with bone-marrow derived hematopoietic cells. It is noted that the examples of the present application have also been performed with bone marrow derived hematopoietic cells. In addition D2 discloses on p. 1652 (column 2, § 1) that all MMH lines supported hematopoiesis from fetal liver or bone marrow hematopoietic cell progenitors and precursors. D2 thus also discloses the coculture of MMH cells with bone-marrow derived hematopoietic cells, which thus includes adult mammalian cells.
It is not directly evident from the passage on p. 1653 (§ 2) relating to experiments with the microporous transwell insert and with the MMH-conditioned medium, whether the latter experiments were also performed with bone-marrow derived cells. But even if this would not be the case, then the disclosure of D2 (relating to successful coculture of MMH cells with bone marrow derived hematopoietic cells) would render the use of the MMH-conditioned medium to the culture of adult cells not inventive. In other words: the disclosure that adult cells can be co-cultured with MMH cells would render it obvious that adult cells can also be cultured in MMH-conditioned medium, in analogy to the results with fetal cells cultured in MMH-conditioned medium. Therefore

no inventive step can be recognized for the use of the MMH-conditioned medium for the culture of adult cells.

1.7 In addition, it is noted that D2 discloses on p. 1653 (§ 2) that for long-term maintenance (in contrast to differentiation) of hematopoietic progenitor cells a **direct contact** between MMH cells and hematopoietic progenitor cells is required. It is further noted that the present application does not provide any experimental evidence for long-term maintenance of hematopoietic cells in MMH-conditioned medium (all examples relate only to expansion and differentiation). Therefore the ED considers that the present invention does not solve the problem with respect to the embodiment of 'maintenance'.

2. Industrial applicability (Article 33(4) PCT)

The subject matter of claims 1-30 appears to be industrially applicable.

NEW SET OF CLAIMS

1. Culture medium conditioned by cytokines and soluble factors released by immortalized untransformed hepatocytes that are differentiated, polarized epithelial cells; said medium being characterized in that it is free from conditioning cells, when used, permitting the maintenance, proliferation and differentiation of adult mammalian cells.

2. Culture medium according to claim 1 wherein said hepatocytes are murine MMH cells.

3. Culture medium according to claims 1-2 wherein said MMH cells are genetically modified.

4. Culture medium according to claims 1-3 wherein said cultured mammalian cells are cord-blood stem cells.

5. Culture medium according to claims 1-3 wherein said cultured mammalian cells are non human embryonic stem cells or adult stem cells including human.

6. Culture medium according to claims 1-3 wherein said mammalian cells are endodermal, ectodermal and mesodermal or their adult progenitor and stem cells.

7. Culture medium according to claims 1-6 characterized for further comprising at least one biological molecule selected from the group consisting of proteins, glycoproteins, lipoproteins, carbohydrates, lipids, glycolipids, peptides, antibodies, cytokines, hormones and enzymes.

8. Culture medium according to claims 1-6 further characterized for being depleted for at least one biological molecule selected from the group consisting of: ~~proteins, glycoproteins, lipoproteins, carbohydrates, lipids, glycolipids, peptides,~~ antibodies, cytokines, hormones and enzymes.

9. Culture medium according to claims 1-6 wherein said untransformed hepatocytes are genetically modified in order to express at least one specific biological factor selected from the group of: proteins, glycoproteins, lipoproteins, carbohydrates, lipids, glycolipids, peptide, antibodies, cytokines, hormones and enzymes.

10. Culture medium according to claims 1-9 in form of a solid, a lyophilized, a powder, a gel, a film, or a freeze-dried compound.

11. Culture medium according to claims 4-6 wherein the maintenance, the

proliferation and the differentiation of mammalian cells is performed in order to further condition the MMH-conditioned medium.

12. Process for production of a culture medium comprising the steps of incubating immortalized untransformed hepatocytes that are differentiated, polarized epithelial cells in a culture medium for at least 2 hours and separating said hepatocytes before the use for maintenance, proliferation and differentiation of adult mammalian cells.

13. Process according to claim 12 wherein the separation step is performed by filtration or by centrifugation.

14. Process according to claims 12-13 wherein said culture medium is RPMI, Ham's F12, Dulbecco's Modified Eagle's Medium (DMEM), RPMI 1640, Iscove's, McCoy's.

15. Process according to claims 12-14 wherein the cells grow in culture either in suspension or in adherence to an extracellular matrix, as monolayers or three-dimensionally.

16. Process according to claim 15 wherein the matrix is solid, such as plastic, or semisolid like a gels, such as collagen, gelatin or agar.

17. Mammalian cells treated with the conditioned medium according to claims 1-11 to be used in the medical field.

18. Mammalian cells according to claims 1-11 to be used for cellular transplantation protocols.

19. Mammalian cells according to claims 1-19 to be subjected to genetic engineering.

20. Mammalian cells according to claims 1-19 to be used for the production of biological molecules.

21. Pharmaceutical composition comprising the mammalian cells according to claims 17-21 to be used in the medical field.

22. Pharmaceutical composition comprising the mammalian cells according to claims 17-21 to be used in cellular therapy protocols

23. Use of the conditioned medium according to claims 1-11 for the preparation of a culture medium for growing, expand, maintain and /or differentiate isolated

adult mammalian cells *in vitro*.

24. Use according to claim 23 wherein said isolated cells are cord-blood stem cells.

25. Use according to claim 23 wherein said isolated cells are non human embrional stem cells or adult stem cells.

26. Use according to claim 23 wherein said cells are endodermal, ectodermal and mesodermal and their adult progenitor and stem cells.

27. Use according to claim 23 wherein said cells are NK cells.

28. Use according to claim 23 wherein said cells are dendritic cells.

29. Use according to claim 23 wherein said cells are endothelial cells.

30. Kit for maintenance, proliferation and differentiation of adult mammalian cells, said kit comprising the culture medium according to claims 1-11 together with laboratory means and instructions for use.

Box No. VIII (iv) DECLARATION: INVENTORSHIP (only for the purposes of the designation of the United States of America)

The declaration must conform to the following standardized wording provided for in Section 214; see Notes to Boxes Nos. VIII, VIII (i) to (v) (in general) and the specific Notes to Box No. VIII (iv). If this Box is not used, this sheet should not be included in the request.

Declaration of inventorship (Rules 4.17(iv) and 51*bis*.1(a)(iv)) for the purposes of the designation of the United States of America:

I hereby declare that I believe I am the original, first and sole (if only one inventor is listed below) or joint (if more than one inventor is listed below) inventor of the subject matter which is claimed and for which a patent is sought.

This declaration is directed to the international application of which it forms a part (if filing declaration with application).

This declaration is directed to international application No. PCT/EP2004/051758 (if furnishing declaration pursuant to Rule 26*ter*).

I hereby declare that my residence, mailing address, and citizenship are as stated next to my name.

I hereby state that I have reviewed and understand the contents of the above-identified international application, including the claims of said application. I have identified in the request of said application, in compliance with PCT Rule 4.10, any claim to foreign priority, and I have identified below, under the heading "Prior Applications," by application number, country or Member of the World Trade Organization, day, month and year of filing, any application for a patent or inventor's certificate filed in a country other than the United States of America, including any PCT international application designating at least one country other than the United States of America, having a filing date before that of the application on which foreign priority is claimed.

Prior Applications: Italy Application No. RM2003A000395 filed on 12 August 2003

I hereby acknowledge the duty to disclose information that is known by me to be material to patentability as defined by 37 C.F.R. § 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the PCT international filing date of the continuation-in-part application.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name: Veronica BORDONI

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Mailing Address: Via Avicenna 19 - 00146 ROMA - ITALY

Citizenship: Italian

Inventor's Signature: Veronica Bordoni
(if not contained in the request, or if declaration is corrected or added under Rule 26*ter* after the filing of the international application. The signature must be that of the inventor, not that of the agent)

Date: 4 October 2004
(of signature which is not contained in the request, or of the declaration that is corrected or added under Rule 26*ter* after the filing of the international application)

Name: Tonino ALONZI

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Inventor's Signature:
(if not contained in the request, or if declaration is corrected or added under Rule 26*ter* after the filing of the international application. The signature must be that of the inventor, not that of the agent)

Date: 4 October 2004
(of signature which is not contained in the request, or of the declaration that is corrected or added under Rule 26*ter* after the filing of the international application)

☒ This declaration is continued on the following sheet, "Continuation of Box No. VIII (iv)".

Continuation of Box No. VIII (i) to (v) DECLARATION

If the space is insufficient in any of Boxes Nos. VIII (i) to (v) to furnish all the information, including in the case where more than two inventors are to be named in Box No. VIII (iv), in such case, write "Continuation of Box No. VIII ..." (indicate the item number of the Box) and furnish the information in the same manner as required for the purposes of the Box in which the space was insufficient. If additional space is needed in respect of two or more declarations, a separate continuation box must be used for each such declaration. If this Box is not used, this sheet should not be included in the request.

CONTINUATION OF BOX NO. VIII (iv) DECLARATION: INVENTORSHIP

Name: Marco TRIPODI

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Name:

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Inventor's Signature: .....

Date:

Name:

Residence:
(city and either US state, if applicable, or country)

Mailing Address:

Citizenship:

Inventor's Signature: .....

Date: